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Fine Structure of the Dorsal Tongue Surface in the Japanese Toad, *Bufo japonicus* (Anura, Bufonidae)

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ABSTRACT—The ultrastructure of the epithelial cells and sensory organs of the dorsal surface of the tongue of *Bufo japonicus* were investigated by scanning electron microscopy. The specimens were prepared using a method designed to remove the extracellular material which normally adheres to the tongue's surface. Irregular undulant structures, or ridge-like papillae, which correspond to the filiform papillae of *Rana,* were compactly distributed over almost all of the dorsal surface of the tongue, while fungiform papillae were scattered amongst these ridge-like papillae. A round sensory disc was located on the top of each fungiform papilla. Latticework, which represented the outline of the boundary of each cell, was visible on the surface of each sensory disc. At higher magnification, we observed that the surface of almost every sensory disc was covered with a honeycomb structure, while a small number of cells with microvilli on their surfaces were scattered amongst them. Each sensory disc was encircled by a thin band of non-ciliated cells. Microridges were widely distributed on the epithelial cell surface of the ridge-like papillae. The observed micro-ornamentation of the lingual structure with its microridges and honeycomb structures may be related to the retention of mucus on the surface of the anuran tongue.

INTRODUCTION

Filiform and fungiform papillae are distributed on the dorsal surface of the anuran tongue. A round sensory disc is located on the top of each fungiform papilla. Electron microscopic studies of the structure of the sensory discs include those of Graziadei [1], Graziadei and DeHan [2], Düring and Andres [3] and Gubo *et al.* [4], all of whom reported that the entire surface of each sensory disc of the tongue is covered with microvilli. However, Jaeger and Hillman [5] described the cytoplasmic ridges of the associated cells of the sensory disc, as well as interspersed cells with microvilli. More recent studies [6, 7] have revealed that when mucus is almost completely removed, most of the surface of the sensory disc is covered with a honeycomb structure. It is possible that these conflicting observations reflect interspecific variations among the anuran species examined. We have attempted to ascertain whether the honeycomb structure of the sensory disc occurs in the genus *Bufo* (Bufonidae) as well as in the frogs of the genus *Rana* (Ranidae).

In all but the most recent reports on studies of the anuran tongue [4, 6, 7], the fine structure of the surface of the filiform papillar cells has been neglected. The present study examines the structure of the surface of the ridge-like papillae in *Bufo japonicus,* since these structures may be analogous to the filiform papillae of *Rana.*

MATERIALS AND METHODS

Tongues from four male and three female adult japanese toads, *Bufo japonicus,* were used in the present study. The toads were perfused from the heart with Karnovsky fixative [8] under anesthesia with MS-222. The tongues were then removed and fixation was continued by immersion in the same solution. After rinsing in 0.1 M cacodylate buffer, several specimens were postfixed in phosphate-buffered 1% osmium tetroxide solution [9] at 37°C for 2 hr and then treated with 8 N hydrochloric acid at 60°C for 30 min to remove

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extracellular substances by acid hydrolysis. For use as controls, a few specimens were not subjected to the postfixation and treatment with acid. All of the specimens were then dehydrated, critical-point dried and coated by gold-ion sputtering. Finally, the specimens were examied under a scanning electron microscope (Hitachi S-500, S-800).

RESULTS

When specimens were postfixed in 1% osmium

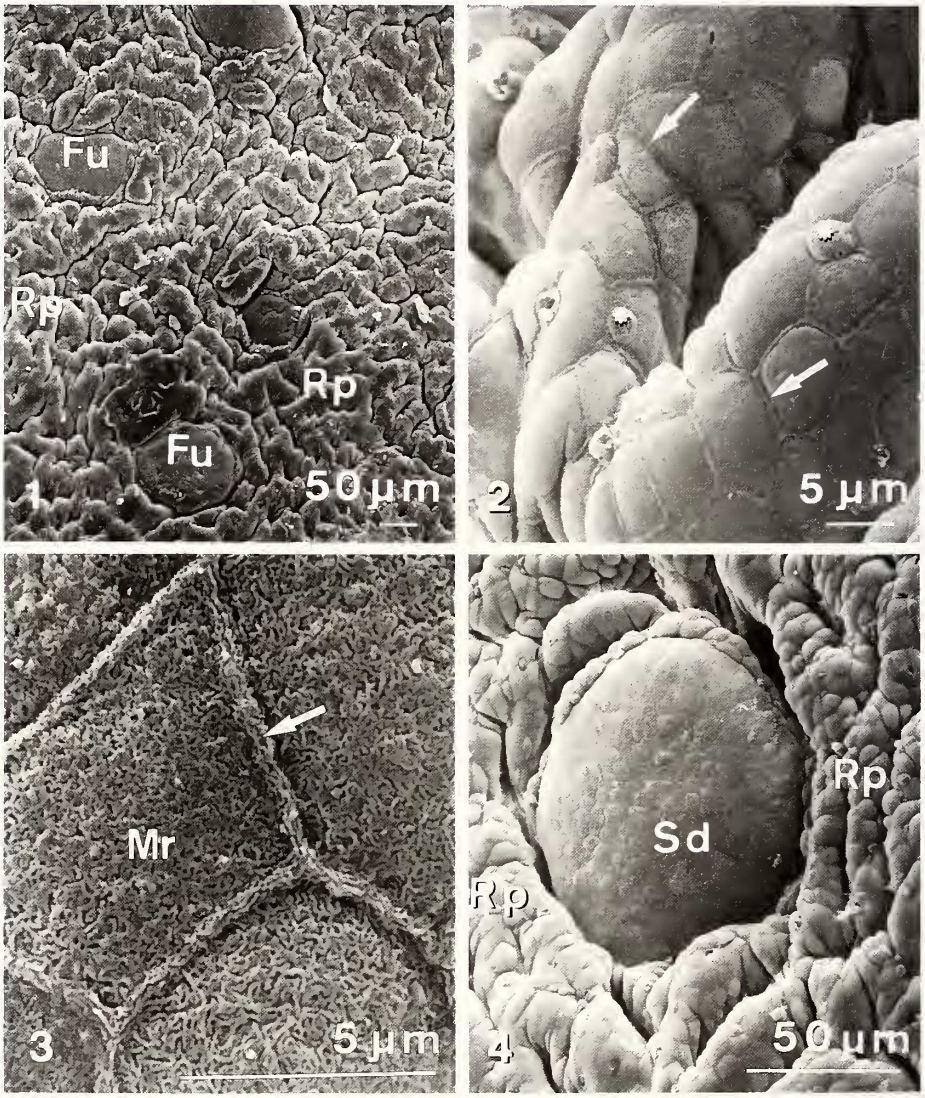

FIG. 1. Scanning electron micrograph of the central dorsal surface of the tongue of *Bufo japonicus*. Rp: ridge-like papillae, Fu: fungiform papillae.

FIG. 2. Ridge-like papillae from *Bufo japonicus*. Arrows show elevated intercellular borders. Asterisks indicate structures related to mucous secretion.

FIG. 3. Higher magnification of polygonal, non-ciliated cells of the ridge-like papilla in *Bufo japonicus*. Mr: microridges. Arrow shows elevated intercellular borders.

FIG. 4. Fungiform papillae from *Bufo japonicus*. Sd: sensory disc, Rp: ridge-like papillae.

tetroxide and treated with 8 N hydrochloric acid, extracellular substances were almost completely removed.

Irregular, undulant structures or ridge-like papillae are distributed in a compact arrangement over the entire dorsum of the tongue, except for its anterior margin. Fungiform papillae, 100–150 μm in diameter, are scattered among the ridge-like papillae (Fig. 1).

The ridge-like papillae are 20–50 μm in width. They are covered with polygonal, non-ciliated cells, the borders of which are elevated (Fig. 2,

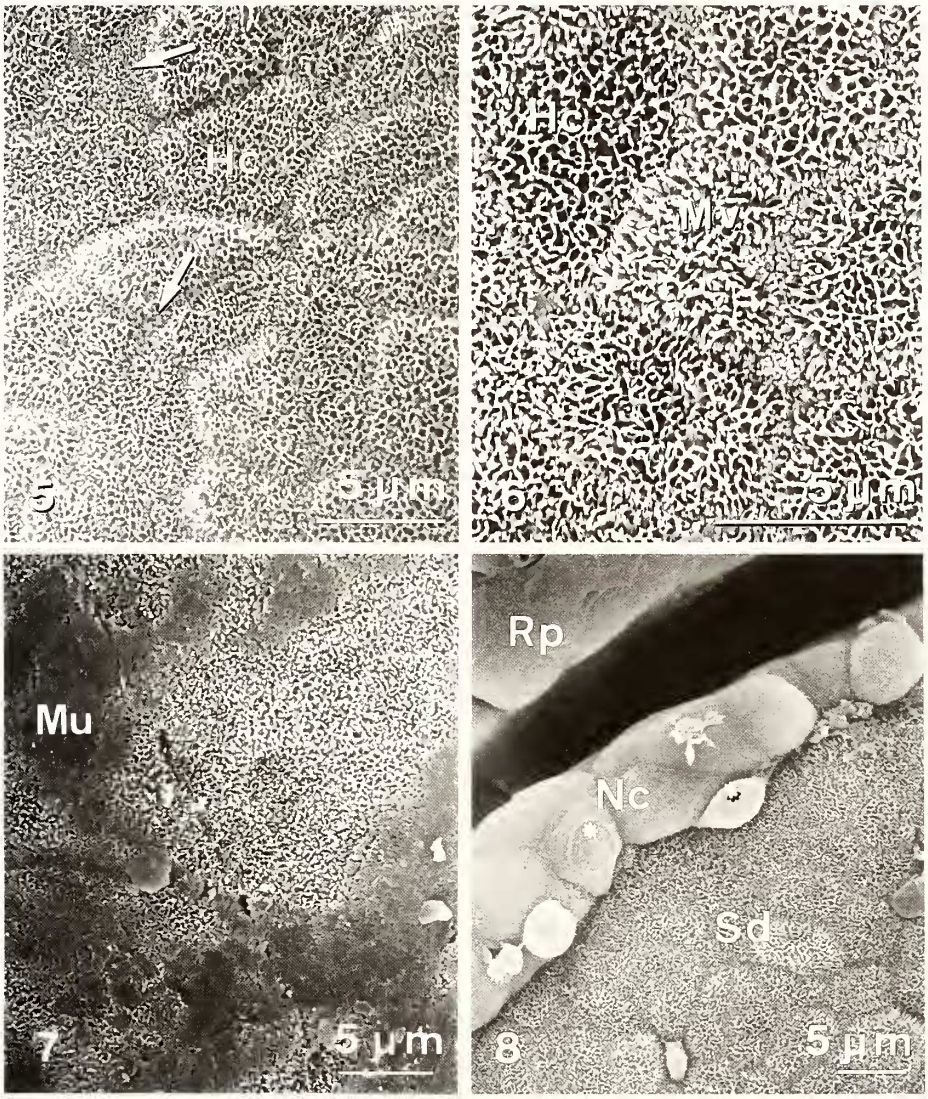

Fig. 5. The surface of a sensory disc from *Bufo japonicus*. Hc: honeycomb structure. Arrows indicate microvilli.

Fig. 6. Higher magnification of a sensory disc of the tongue of *Bufo japonicus*. Hc: honeycomb structure, Mv: microvilli.

Fig. 7. Higher magnification of a sensory disc of the tongue of *Bufo japonicus*, without postfixation with osmium tetroxide and acid treatment. Mu: piled mucus.

Fig. 8. Boundary region of a sensory disc and non-ciliated cell. Sd: sensory disc, Nc: non-ciliated cells, Rp: ridge-like papillae. Asterisks indicate structures related to secretion of mucus.

arrow). Mucus-secreting cells are scattered among these epithelial cells (Fig. 2, asterisks). At higher magnification (Fig. 3), fine plications, or microridges, are densely distributed on the surfaces of non-ciliated cells. The elevated intercellular borders are composed of bundles of such plications. In specimens which were not postfixed with osmium tetroxide, the surfaces of the ridge-like papillae are obscured by mucus.

A sensory disc is located in the central area of the top of each fungiform papilla (Fig. 4). The surface of the disc has a latticework pattern which reflects the boundaries of the cells on the surface of the papilla. At higher magnifications of the

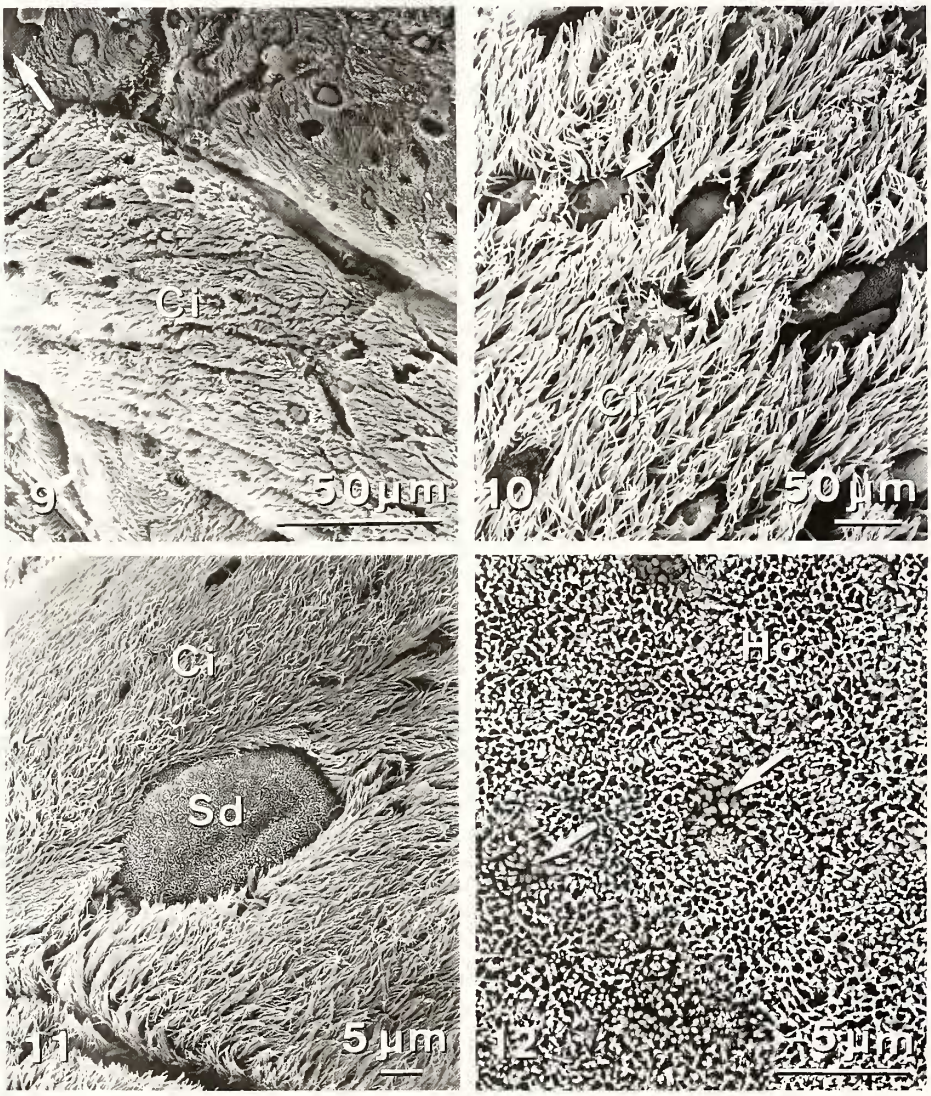

Fig. 9. Long ridges in the area near the apex. Ci: ciliated cells.

Fig. 10. Higher magnification of the long ridges in the area near the apex. Ci: ciliated cells. Arrow indicates the area without cilia.

Fig. 11. Sensory disc (Sd) in the area near the apex. Ci: ciliated cells.

Fig. 12. Higher magnification of a sensory disc in the area near the apex. Arrows indicate micorovilli. Hc: honeycomb structure.

sensory disc (Fig. 5), the cell surfaces resemble a honeycomb. Many processes, which are 0.1–0.3 μm long, are recognizable on the honeycomb-like structure. A few cells with microvilli are present among the honeycomb-like cells (Figs. 5 and 6). In specimens not subjected to postfixation and acid treatment, mucus forms a thin covering over the honeycomb framework of the cells (Fig. 7). Each sensory disc is surrounded by non-ciliated cells, which appear to be the same as those that form the ridge-like papillae (Fig. 8).

A series of long ridges about 1 mm in width are present on the anterior margin of the dorsal surface of the tongue in parallel with its anterior edge (Fig. 9). The surfaces of the long ridges are covered almost entirely by ciliated cells. Ridge-like papillae composed of non-ciliated cells are not found in this area. Non-ciliated areas are scattered on the surface of these ciliated cells (Fig. 10, arrow). Structures similar to the sensory discs on the top of fungiform papillae occur among the ciliated cells on the surfaces of these ridges (Fig. 11). At higher magnification, these discs appear to be identical to the discs described above (Fig. 12, compare with Fig. 6).

DISCUSSION

In several earlier reports [1–3], the surface of the sensory discs of the frogs, *Rana pipiens*, *Rana esculenta* and *Rana temporaria*, was described as being extensively covered with microvilli. In contrast, Jaeger and Hillman [5] described the cytoplasmic ridges of the associated cells of the sensory disc, as well as interspersed cells with microvilli in *Rana catesbeiana* and *Hyla arborea*. In our specimens of *Bufo japonicus* from which the mucus was removed, the greater part of the surface of each sensory disc was found to be covered with a latticework pattern similar to that which was demonstrated by us in two species of *Rana* namely *Rana catesbeiana* [6] and *Rana nigromaculata* [7]. As in *Rana*, most of the surface of each lingual sensory disc of *Bufo japonicus* is covered with a honeycomb-like texture of cell surfaces, which originate from "associated cells" designated by Graziadei and DeHan [2]. The honeycomb-like texture is completely coincident with the "cytoplasmic ridges" described by Jaeger and Hillman [5]. In the present study, thin processes were also recognized on the surface of the honeycomb-like textures, just as in the observation by Jaeger and Hillman [5]. They identified these structures as microvilli. However, we feel that these protrusions are too small to be considered microvilli. Microvilli, which are located between these "associated cells", may derive from the "sensory cell" described by Key [10]. The honeycomb-like structures may play a role in the retention of water and other mucous fluid on the surface of the sensory disc. In addition, the possibility that the honeycomb-like structure has the same function as the taste hair is undeniable. On the other hand, it has been shown in a previous study [7] and in the present study that, when the mucus which covers the lingual surface is not completely removed, the remaining mucus may be transformed into various crystal structures during the drying of the specimens.

Among anurans, we were able to recognize some morphological differences in the tongues of two species of *Rana* [6, 7] and now can compare them to a species of *Bufo*, *Bufo japonicus*. In *Bufo japonicus*, the epithelium formed many ridge-like papillae, and the pores related to the secretion of mucus were not obvious on its surface, while in the two species of *Rana*, the epithelial surface formed many filiform papillae and there were many pores on its surface [6, 7]. In addition in *Bufo japonicus*, no ciliated cells were observed on the surface of the ridge-like papillae and on the surrounding areas of the sensory disc, while, in *Rana*, many ciliated cells were seen on the surface of the filiform papillae and the surrounding areas of the sensory discs. Structures and surface features which were somewhat similar to the ridge-like papillae in *Bufo*, were shown by Gubo *et al.* [4] in *Bombina variegata*. However, they did not describe these papillae in detail. The differences between anurans belonging to the varied genuses may be ascribed to the local differentiation of function of the lingual mucosa.

In *Rana*, microridges were reported to be widely distributed on the non-ciliated cells of the filiform papillae [6, 7]. The present study of *Bufo japonicus* revealed that microridges are also present on

the non-ciliated cells of the ridge-like papillae. In our study of *Rana nigromaculata* using transmission electron microscopy [11], it appeared that a large fraction of the filiform papillar epithelial cells had both microridges on the free surface of cells and cellular processes on the surfaces which faced adjacent cells. Thus, the microridges may be the result of an altered pattern of arrangement of these cellular processes, which function as connecting structures between adjacent cells [12]. As suggested by Sperry and Wassersug [13], microridges may be important for holding mucus on the surface of the cells. Thus, the lingual microridges in *Bufo* may function to retain mucus on the dorsal surface of the tongue.

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